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Telomere Length Shortening Is Associated With Disease Evolution in Chronic Myelogenous Leukemia

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We studied telomere length in the peripheral blood leukocyte samples of a large group of patients with chronic myelogenous leukemia (CML) by Southern blot hybridization using the $(TTAGGG)_4$ probe. The average telomere length expressed as the peak telomere repeat array (TRA) of the peripheral blood samples obtained from a group of 34 healthy age-matched controls ranged between 7.6 and 10.0 kb and the mean peak TRA was 8.7 kb. Forty-one patients in the chronic phase of CML were studied; 32/41 (78%) showed telomere reduction (<7.6 kb) relative to age-matched controls and the mean peak TRA was 6.4 kb (range 4.0–10.6 kb). Serial samples were analysed from 12 patients at both chronic phase and during disease progression. The leukocyte DNA of all 12 patients in accelerated phase and/or blast crisis showed telomere reduction relative to age-matched controls and the mean peak TRA was 4.1 kb (range 3.0–5.4 kb). The peak TRA in the accelerated or blast phase was reduced compared with the corresponding paired sample in the chronic phase in all cases studied. These data show that a marked reduction in telomere length is associated with disease progression in CML. Am. J. Hematol. 61:5–9, 1999.

Key words: chronic myelogenous leukemia; telomere; leukemic transformation; blast crisis; accelerated phase

INTRODUCTION

Human chromosomes terminate with the simple telomere repeat $(TTAGGG)_n$ [1]. Telomeres have a number of important functions including the protection of chromosomes from end-to-end fusion, degradation, and rearrangement [1,2]. Telomeric sequences are synthesized by telomerase, a ribonucleoprotein enzyme that extends the 3′ end of telomeres [3,4].

In humans, germline cells express telomerase and retain their telomere length [5]. Somatic cells do not express telomerase and their telomeres shorten with each division [5]. A reduction in telomere length has been reported in a wide range of human tumors, including both solid tumors and hematological malignancies [6,7].

There is much interest in the determination of those features associated with progression of chronic myelogenous leukemia (CML). CML usually presents in a chronic phase, which is controlled by therapy. It progresses into an accelerated phase, the duration of which is generally less than one to one and a half years [8,9]. The accelerated phase is followed by a blastic phase (blast crisis) resulting in the patients death within 3 to 6 months [8]. We have studied telomere length in CML to determine whether a change in telomere length is associated with disease evolution.

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TABLE I. Peak TRA (kb) of the Peripheral Blood Samples Obtained From a Group of CML Patients During the Chronic Phase of the Disease*

Patient no.	Age	Time from diagnosis (months)	Sokal score at diagnosis	Peak TRA (kb)
1	44	38	1.53	5.8
2	44	3	0.57	6.2
3	54	43	3.64	5.9
4	60	115	2.21	5.9
5	68	41	4.16	5.9
6	39	32	0.94	5.8
7	24	26	4.38	6.6
8	67	0	2.63	8.1
9	42	20	1.02	8.1
10	40	41	2.63	10.5
11	53	23	2.67	7.0
12	27	1	0.59	8.0
13	41	22	5.23	5.5
14	31	1	1.4	6.5
15	56	1	0.83	6.5
16	30	3	0.35	7.6
17	62	23	n/a	5.2
18	69	4	0.82	6.2
19	24	24	1.05	5.7
20	32	0	0.42	5.2
21	59	21	0.88	8.1
22	19	6	3.83	8.5
23	66	1	1.83	5.6
24	37	0	1.66	4.0
25	49	1	1.81	10.6
26	35	0	0.42	10.6
27	46	0	n/a	5.1
28	25	6	0.63	5.4
29	36	1	0.31	5.4
30	45	20	3.69	6.3
31	67	28	1.92	7.5
32	56	38	0.98	5.9
33	24	0	n/a	4.8
34	67	0	n/a	5.5
35	57	0	n/a	4.1
36	22	0	n/a	7.1
37	27	20	0.88	5.6
38	80	3	2.31	6.4
39	28	120	0.73	4.0
40	20	1	4.86	4.5
41	72	0	1.23	7.0

*TRA, telomere repeat array; CML, chronic myelogenous leukemia; n/a, data not available. The Sokal score at diagnosis, patient age at diagnosis, and time from diagnosis that each peripheral blood sample was taken are also shown.

MATERIALS AND METHODS

Forty-one patients with CML were included in the study. The 41 patients were classified as being in the chronic phase of CML at diagnosis. The age range of the 41 patients at diagnosis was 20–80 years and the mean age was 45 years. Peripheral blood leukocyte specimens were obtained from each patient in the chronic phase of CML. The diagnostic Sokal score was calculated for each patient [10]. During the course of this investigation 12 of the 41 patients progressed to accelerated phase and/or blast crisis. Patients were deemed to be in accelerated phase according to previously published definitions of accelerated phase [8]. Blastic phase CML was diagnosed when patients showed 20% or more blasts in the marrow or peripheral blood [10]. Serial peripheral blood samples were obtained from these patients during the progression of their disease. Peripheral blood samples were also obtained from 34 normal healthy age-matched controls (age range 22–81 years, mean age 49 years).

High molecular weight DNA was obtained by phenol/chloroform extraction using standard methods [11] from the peripheral blood leukocytes from the patients with CML and from the peripheral blood leukocytes from nor-

TABLE II. Peak TRA (kb) of a Group of CML Patients During Disease Evolution*

Patient no.	Chronic phase		Accelerated phase		Blast crisis	
	TRA	BC (%)	TRA	BC (%)	TRA	BC (%)
33	6.3	0	—	—	4.7	49
34	7.5	0	5.4	0	—	—
35	5.9	0	4.4	0	—	—
36	4.8	2	—	—	3.9	23
37	5.5	1	—	—	3.3	23
38	4.1	1	3.8	19	—	—
39	7.1	1	5.0	1	—	—
40	5.6	0	4.8	2	—	—
41	6.4	0	3.5	11	3.1	51
42	4.0	0	—	—	3.2	75
43	4.5	0	4.0	0	3.0	29
44	7.0	0	5.2	7	—	—

*TRA, telomere repeat array; BC, peripheral blood blast count; —, sample not available for analysis (ie, patient not yet progressed to blast crisis from accelerated phase or progressed from chronic phase directly to blast crisis). DNA extracted from serial peripheral blood samples was analysed in each case.

mal healthy individuals (age-matched controls). To ensure that there was no evidence of DNA degradation the integrity of each of the undigested DNA samples was checked by electrophoresis through 1% agarose gels. Ten micrograms of DNA digested with the restriction enzyme *Hinf*I was size fractionated by electrophoresis through 0.8% agarose gels. The DNA was transferred to Hybond N (Amersham Int, Amersham, U.K.) according to standard procedures for Southern blotting [10]. The filters were prehybridized in 5 × SSC, 4 × Denhardts' solution, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA for 2–4 hr at 65°C. The filters were hybridized to a 3′-^{32}p labelled $(TTAGGG)_4$ telomeric probe in 5 × SSC for 16–24 hr at 50°C [12]. The filters were washed in 4 × SSC for 30–60 min and autoradiographed between intensifying screens at −70°C for 2–7 days [12]. The telomere lengths were measured with an LKB Ultrascan XL densitometer, with the peak of telomere length in kilobases (peak telomere repeat array; peak TRA) taken as the average telomere length in each patient [13]. The telomeric repeat analysis was performed with each DNA sample on at least two separate occasions.

RESULTS

The peak TRA of the peripheral blood samples obtained from a group of 34 healthy age-matched controls (age range 22–81 years, mean age 49 years) ranged between 7.6 and 10.0 kb and the mean peak TRA was 8.7 kb. These control values are consistent with data from other groups concerning telomere length in human leukocytes within this age range [6]. The lowest TRA value observed in age-matched controls was 7.6 kb. A TRA of less than 7.6 kb was therefore judged to indicate a reduction in telomere length. Similar lower limits have been set by others [7,14]. Forty-one patients in the chronic phase of the disease were studied. The peak TRA of the peripheral blood samples obtained from this group are shown in Table I. 32/41 (78%) patients in chronic phase showed telomere reduction relative to age-matched controls and the mean peak TRA was 6.4 kb (range 4.0–10.6 kb). Twelve patients progressed to accelerated phase and/or blast crisis during the course of this study. Serial samples were obtained from each case and telomeric repeat analysis performed on all samples. The leukocyte DNA of all 12 patients in accelerated phase and/or blast crisis showed telomere reduction relative to age-matched controls and the mean peak TRA was 4.1 kb (range 3.0–5.4 kb) (see Table II). In the serial study we found that the peak TRA in the accelerated or blast phase was reduced compared with the corresponding paired sample in the chronic phase in all cases studied (see Table II and Fig. 1A and B).

The relationship between peripheral blast percentage and TRA was examined through the calculation of the correlation coefficient. The statistical significance of the calculated coefficient was determined using standard statistical tables [15]. The relationship of peripheral blood blast percentage to TRA is outlined in Table II. There is no statistically significant correlation between blast count and telomere length in either accelerated phase ($r = -0.552$) or blast crisis ($r = -0.0348$). Four patients (nos. 31, 32, 37, and 40) displayed a reduction in telomere length between chronic phase and accelerated phase without any increase in the blast count.

Six patients had a blast crisis; these were all myeloid in nature with the exception of one patient (patient 36) who had a lymphoid crisis.

DISCUSSION

We examined telomere length in a large group of patients with CML. DNA obtained from the peripheral blood leukocytes of 41 patients with CML and 34 age-matched healthy controls was subjected to telomeric repeat analysis by Southern blot hybridization using the $(TTAGGG)_4$ telomeric probe. The average telomere length expressed as the mean peak TRA of the age-matched controls was 8.6 kb. Each of the 41 patients had chronic phase CML at diagnosis and the mean peak TRA was 6.4 kb. Thirty-two of forty-one (78%) of these patients showed telomere reduction (<7.6 kb) relative to age-matched controls. This finding is consistent with recent data from Ohyashiki et al. [16] who found that telomere length in the chronic phase of CML was shortened significantly compared with age-matched controls.

The main aim of this study was to determine whether a reduction in telomere length is associated with disease evolution in CML. Serial samples were analysed from 12

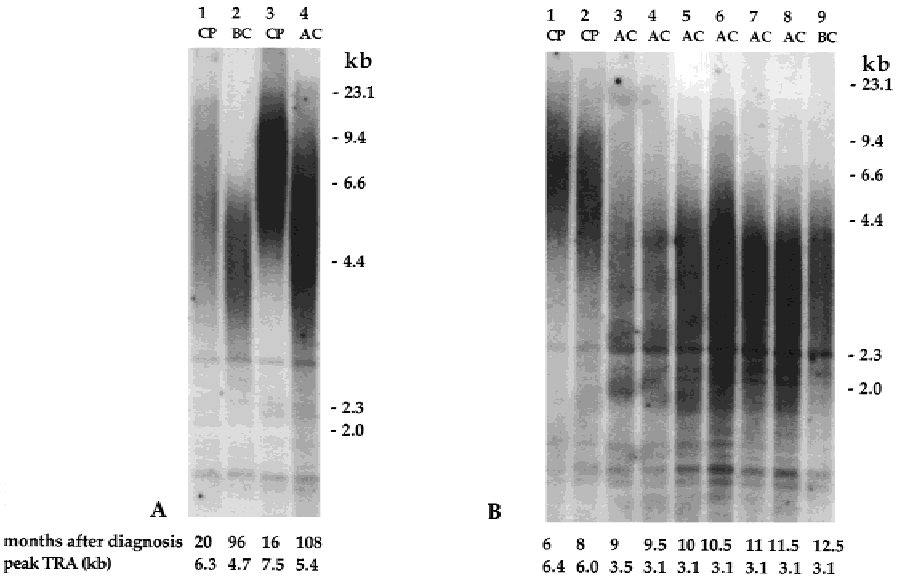

Fig. 1. Southern blot analysis of telomere length using the $(TTAGGG)_4$ probe hybridized against *Hin*fI digested DNA extracted from the peripheral blood samples from representative CML patients during disease evolution. Serial peripheral blood samples were analysed in each case. A: Tracks 1–2 (paired samples) show DNA obtained from patient no. 33; tracks 3–4 (paired samples) show DNA obtained from patient no. 34. B: Tracks 1–9 show DNA (serial samples) obtained from patient no. 41. Note: marked reduction in telomere length in accelerated phase and/or blast crisis. CP, DNA obtained from peripheral blood samples in chronic phase; AC, DNA obtained from peripheral blood samples in accelerated phase; BC, DNA obtained from peripheral blood sample in blast crisis. Approximate position of the molecular weight markers in kilobase pairs (kb), is shown on the right hand side.

patients at both chronic phase and accelerated phase and/or blast crisis. All 12 cases showed telomere reduction relative to age-matched controls in accelerated or blast phase and the mean peak TRA was 4.1 kb. There was no statistically significant correlation between peripheral blood blast percentage and TRA in either accelerated phase or blast crisis. Obviously, the numbers of patients tested are too small to definitively exclude a relationship between blast count and TRA; a trend towards a higher blast count and shorter TRA as the disease progresses may be expected. It is interesting to note, however, that four patients demonstrated a reduction in telomere length in accelerated compared with chronic phase without any recorded rise in blast count. We therefore feel it unlikely that TRA reduction is simply a reflection of rising blast count per se.

In the serial study we observed that the peak TRA in the accelerated or blast phase was reduced compared with the corresponding paired sample in the chronic phase in all cases studied. Thus, a marked reduction in telomere length seems to be associated with disease progression in CML. We were fortunate to have obtained a large number of serial samples from some patients during disease progression and were therefore able to monitor telomere length changes in detail during disease evolution. A marked reduction in telomere length during transformation to acute leukemia was clearly apparent from this serial study. (see Table II, Fig. 1B.)

A reduction in telomere length has been shown to be associated with disease progression in certain other hematological malignancies. We and others have shown that a marked reduction in telomere length in MDS is often associated with leukemic transformation [13,14]. Similarly, an association between a reduction in telomere length and the progression of chronic lymphocytic leukemia has been demonstrated [17].

This study has shown that telomere shortening occurs in some patients at accelerated phase as well as blast crisis of CML. This observation is of particular interest as it suggests that a marked reduction in telomere length may be a feature of early leukemic transformation in CML. A large study in which careful serial measurements of telomere length are made in individual patients throughout the entire course of their disease is clearly warranted to assess the precise stage of disease evolution at which these changes take place.

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